

## Electrophoretic Patterns of Seed Proteins in the East Asian *Vicia* Species (Leguminosae) and Their Systematic Utility

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(Received on June 25, 2002)

Electrophoretic patterns of seed proteins of 12 taxa of 9 species of *Vicia* endemic to East Asia were analysed. A total of 53 stable bands was detected. Inter- and intra-specific phylogenetic relationships among these taxa were inferred with a UPGMA dendrogram from Jaccard's similarity matrix of the band patterns. The resultant phylogenetic implications were consistent with those from morphological or DNA data. We suggest that the electrophoretic pattern of seed proteins is useful to clarify inter- and intra-specific phylogenetic relationships among the East Asian endemic taxa of *Vicia*.

**Key words:** electrophoresis, Leguminosae, phylogeny, seed protein, *Vicia*.

### Introduction

Plants of *Vicia* are usually vines with tendrils, while some species endemic to East Asia are erect perennials without tendrils. These erect perennials are *V. bifolia* Nakai, *V. unijuga* A. Br., *V. fauriei* Franch., *V. nipponica* Matsum., and *V. venosa* (Willd. ex Link) Maxim. These five species are similar to each other in floral and pollen morphological features (Endo and Ohashi 1996). They have been considered to be related each other and are included in section Vicilla of subgenus Vicilla in the genus (Kupicha 1976, Endo and Ohashi 1996). To clarify the phylogenetic relationships among such closely related species with morphological data alone is thought to be difficult. For example, *V. bifolia* is characterized by having unijugate leaves and persistent larger bracts. *V. unijuga* has unijugate leaves but deciduous smaller bracts. *V. fauriei* has persistent

larger bracts but multijugate leaves. Consequently, *V. bifolia* had been treated as a variety of *V. unijuga*, *V. unijuga* var. *bracteata* Franch. & Sav., or a variety of *V. fauriei*, *V. fauriei* var. *unijuga* Matsum. (Ohwi 1965). To which species *V. bifolia* is phylogenetically closely related is still in question.

Seok and Choi (1998) investigated relationships among *Vicia bifolia*, *V. unijuga* and *V. venosa* based on random amplified polymorphic DNA markers. They concluded that *V. bifolia*, having unijugate leaves, is more closely related to *V. venosa*, having multijugate ones, than to *V. unijuga*, having unijugate ones. However, phylogenetic relationships among the five East Asian etendrilous species, including *V. fauriei* and *V. nipponica*, are still in question.

Electrophoresis of seed proteins has been used to investigate phylogenetic relation-

ships among species of section *Faba* in *Vicia* (Ladizinsky 1975, Sammour 1989, Przybylska and Zimniak-Przybylska 1995, Zimniak-Przybylska and Przybylska 1995). The results of these studies were congruent with that from DNA analysis (Potokina et al. 1999). These facts mean that the electrophoresis of seed proteins is applicable in studies of phylogenetic relationships among other closely related species in *Vicia*.

Recent studies on phylogenetic relationships among taxa of *Vicia* have been conducted with analyses of limited sets of DNA sequences. We consider that seed protein analysis may show genetic variation less directly but more wholly than the recent studies with the limited sequences of DNA. In this respect, we analysed seed proteins for phylogenetic applicability in East Asian etendrilous *Vicia* in the light of the result of DNA analysis by Seok and Choi (1998).

### Materials and Methods

We examined seed proteins of the five East Asian etendrilous species, mentioned above, of *Vicia*. For comparison with these species, we also examined four East Asian tendrilous species having climbing habit. These etendrilous and tendrilous East Asian species all belong to section *Vicilla*. Their names, collection sites and voucher specimens are listed in Table 1.

Total seed proteins were estimated by one-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Seed protein extracts were prepared by removing the seed coat and soaking 4 mg of homogenizing cotyledon meal in 1 M Tris-HCl pH 6.8 buffer at room temperature. Samples of supernatant obtained under centrifugation at 12,000 g for 10 min were mixed with an equal volume of buffer containing 0.625 M Tris-HCl pH 6.8, 2 % SDS, 5 % mercaptoethanol and 0.01 % bromophenol blue. Polyacrylamide gels were prepared according to Laemmli (1970). The loading

gel had a 5 % acrylamide concentration in a 2.5 M Tris-HCl pH 6.8 with 10 % SDS. The resolving gel was prepared with 10% acrylamide in a 3.5 M Tris-HCl pH 8.8 buffer with 10 % SDS. The electrode buffer was tris-glycine (6.0 g of Tris base, 28.8 g of glycine, 20 ml of 10 % SDS in 2 liters of water, pH 8.3). Samples were run for 3.5 h at 30 mA in a Hiy Kalur apparatus. Polyacrylamide gels were stained with 0.1 % Coomassie Blue for 12 h and then washed with 7 % acetic acid (Methodical Instructions, 1990). The approximate M.R.s of polypeptide bands were estimated by SDS-PAGE using the following standard proteins: bovine serum albumin (67 kDa), egg albumin (45 kDa), chymotrypsinogen A (25 kDa), myoglobin (17.8 kDa) and cytochrome C (12.4 kDa).

Cotyledons of mature seeds of 3–5 individuals of each accession were examined separately. Bulks containing seed protein extracts from all individuals of each accession under study were also analyzed.

Registration of the detected variation was done by numerous side-by-side comparisons of protein extracts to establish an order of electrophoretic mobilities of all the bands recorded (Przybylska and Zimniak-Przybylska 1995). The compared bands were recorded as different if even a slight difference in their electrophoretic mobility proved to be reproducible.

The similarity of the protein portraits of all taxa under study were estimated using phenetic analysis. Gels were scored for presence or absence of specific bands for all accessions investigated.

A Jaccard's similarity index for all pairwise comparisons between accessions was calculated.

A similarity matrix was used to generate a phenogram by the unweighted pair group method with arithmetic averages (UPGMA) using NTSYS-pc version 1.70 (Rohlf 1992).

Table 1. Species name, collection sites, voucher specimens, and abbreviations of the accessions of *Vicia* examined

| Species  | Collection site of the seeds                                   | Voucher specimens                      | Abbreviations | No. <sup>a</sup> |
|--|--|--|---------------|------------------|
| <i>V. amoena</i> Fisch.  | Japan, Iwate Pref., Morioka                                    | N. Tomooka & E. Potokina in 1998 (WIR) | amo 1         | 5                |
| <i>V. amoena</i> Fisch.  | Japan, Iwate Pref., Hanamaki                                   | N. Tomooka & E. Potokina in 1998 (WIR) | amo 2         | 5                |
| <i>V. amoena</i> Fisch.  | Japan, Akita Pref., Kakunodate                                 | N. Tomooka & E. Potokina in 1998 (WIR) | amo 3         | 5                |
| <i>V. amurensis</i> Oetting.   | Japan, Akita Pref., Oga-shi, Goriai                            | Y. Endo 524 (TUS)                      | amu           | 5                |
| <i>V. bifolia</i> Nakai  | Japan, Saitama Pref., Chichibugun, Ogano-machi, Mt. Futagoyama | No voucher specimen                    | bif           | 5                |
| <i>V. fauriei</i> Franch.  | Japan, Yamagata Pref., Oishidamachi                            | Y. Endo 528 (TUS)                      | fau           | 4                |
| <i>V. japonica</i> A. Gray   | Russia, Kurily range, Iturup Isl.                              | A. Stankevich 5080 (WIR)               | jap           | 3                |
| <i>V. nipponica</i> Matsum.  | Japan, Miyagi Pref., Ojyoji                                    | Y. Endo in 1981 (TUS)                  | nip           | 4                |
| <i>V. pseudo-orobus</i> Fisch. & C.A.Mey.  | Japan, Iwate Pref., Mt. Himegamiyama                           | Y. Endo 542 (TUS)                      | ps-or 1       | 4                |
| <i>V. pseudo-orobus</i> Fisch. & C.A.Mey.  | Japan, Aomori Pref.  | N. Tomooka & E. Potokina in 1998 (WIR) | ps-or 2       | 5                |
| <i>V. unijuga</i> A.Br.  | Japan, Miyagi Pref., Sendai                                    | Y. Endo 526 (TUS)                      | uni 1         | 4                |
| <i>V. unijuga</i> A.Br.  | Japan, Kanagawa Pref., Miura Peninsula, Hayama                 | Y. Endo & T. Nemoto in 1984 (TUS)      | uni 2         | 4                |
| <i>V. unijuga</i> A.Br.  | Japan, Iwate Pref., Mt. Himegamiyama                           | Y. Endo 544 (TUS)                      | uni 3         | 4                |
| <i>V. unijuga</i> A.Br.  | Japan, Saitama Pref., Mt. Buko-san                             | Y. Endo 551 (TUS)                      | uni 4         | 4                |
| <i>V. unijuga</i> A.Br.  | Japan, Oita Pref., Yufuin                                      | Y. Endo in 1981 (TUS)                  | uni 5         | 4                |
| <i>V. venosa</i> (Willd. ex Link) Maxim.<br>subsp. <i>cuspidata</i> (Maxim.) Y.Endo & H.Ohashi   | Japan, Nagano Pref., Togakushi                                 | Y. Endo in 1981 (TUS)                  | ven-cus       | 4                |
| <i>V. venosa</i> (Willd. ex Link) Maxim.<br>subsp. <i>cuspidata</i> (Maxim.) Y.Endo & H.Ohashi<br>var. <i>cuspidata</i> Maxim.<br>f. <i>minor</i> (Nakai) Ohwi | Japan, Kumamoto Pref., Aso-gun, Aso-machi                      | Y. Endo 3150 (CBM)                     | ven-cus-m     | 4                |
| <i>V. venosa</i> (Willd. ex Link) Maxim.<br>subsp. <i>cuspidata</i> (Maxim.) Y.Endo & H.Ohashi<br>var. <i>glabristyla</i> Y.Endo & H.Ohashi                    | Japan, Niigata Pref., Itoigawa-shi, Renge-onsen                | Y. Endo 3140 (CBM)                     | ven-gla       | 5                |
| <i>V. venosa</i> (Willd. ex Link) Maxim.<br>subsp. <i>stolonifera</i> Y.Endo & H.Ohashi  | Japan, Kyoto Pref., Kyoto-shi, Kibune                          | Y. Endo 3147 (CBM)                     | ven-sto       | 4                |

<sup>a</sup>No.: Number of seeds examined.

## Results and Discussion

**Total electrophoretic bands recognized:** A total of 53 stable bands across all 19 accessions of 12 taxa of *Vicia* were recognized. These bands are shown in Figs. 1–3. The number of bands in accessions varied between 17 (*V. fauriei*) and 24 (*V. venosa*). Presence or absence of specific bands for all accessions was shown in Table 2. Jaccard's similarity matrix is shown in Table 3.

### Comparison of seed protein electrophoresis data with morphological or DNA data:

The UPGMA dendrogram (Fig. 4) shows that all taxa examined were primarily arranged into two clusters ('a' and 'b'). The 'a' cluster is composed of *Vicia pseudo-orobus*, *V. amoena*, *V. amurensis*, and *V. japonica*,

while the 'b' *V. nipponica*, *V. venosa*, *V. fauriei*, *V. bifolia*, and *V. unijuga*. The species of the 'a' cluster have tendrils and climbing habit, but those of the 'b' cluster have not. Therefore, the result of the electrophoresis is consistent with this morphological grouping.

The electrophoresis indicates that seed protein banding patterns of *Vicia bifolia* are more similar to *V. venosa* than to *V. unijuga* (Fig. 4). This fact may mean that *V. bifolia* is phylogenetically closer to *V. venosa* than to *V. unijuga*. This result is consistent with that of Seok and Choi (1998) based on DNA data and supports their conclusion.

*Vicia amoena*, *V. amurensis* and *V. japonica* are allied to each other (Ohwi 1965), but floral morphological distinctions among

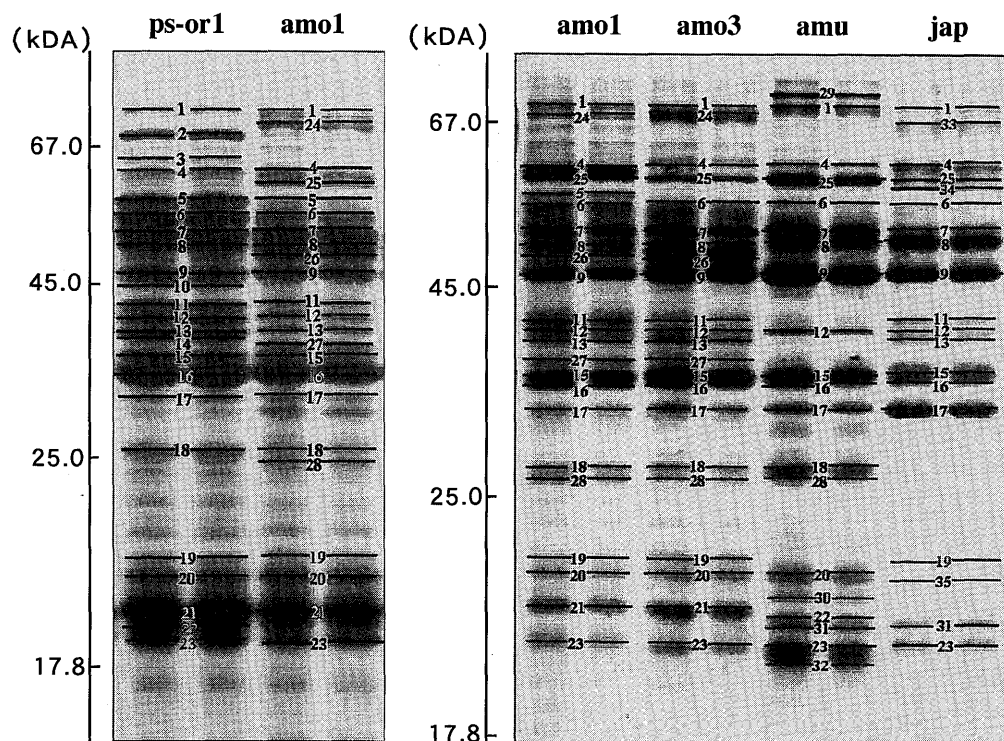


Fig. 1. SDS-PAGE patterns of seed proteins illustrating band patterns detected from five accessions of *Vicia*. The abbreviations of accessions are designated in Table 1.

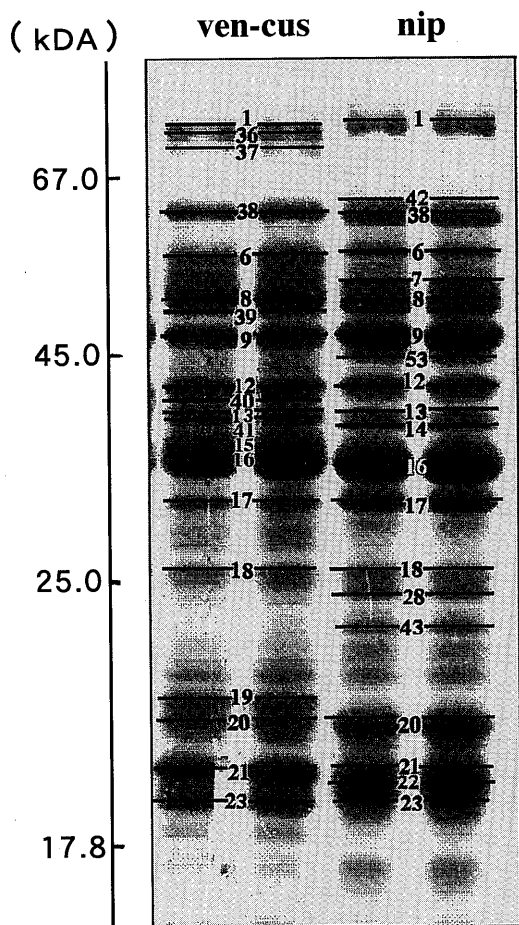


Fig. 2. SDS-PAGE patterns of seed proteins illustrating band patterns detected from two accessions of *Vicia*. The abbreviations of accessions are designated in Table 1.

these species were recently discovered (Endo 1994). Within 'a' cluster, although the electrophoresis indicates the close proximity among them, there is a visible difference among protein portraits of these species (Fig. 2). This result is consistent with the morphological data of Endo (1994).

**Discovered phylogenetic relationships:**  
*Vicia venosa* subsp. *cuspidata* var. *cuspidata*

*f. minor* showed the lowest similarity with other intraspecific taxa of *V. venosa* (Fig. 4). This fact may mean that the three intraspecific taxa, subsp. *cuspidata* var. *cuspidata* f. *cuspidata*, subsp. *cuspidata* var. *glabristyla* and subsp. *stolonifera*, are phylogenetically closely related to each other but far from subsp. *cuspidata* var. *cuspidata* f. *minor*. This presumption is not congruent with the recent taxonomical treatment of the *f. minor*. The forma *minor* generally smaller but thicker leaflets than other intraspecific taxa in the range of variation of *V. venosa* (Endo and Ohashi 1986). Furthermore, the other three intraspecific taxa of *V. venosa* are distributed in Eastern Honshu in Japan and the *f. minor* in Western Honshu and Kyushu (Endo and Ohashi 1986). As the phylogenetic relationships among the intraspecific taxa presumed from seed protein analysis is congruent with their distributional patterns, we consider that *f. minor* is phylogenetically far from the other three intraspecific taxa of *V. venosa* in Japan.

The electrophoresis indicates that seed protein components of *Vicia bifolia* are more similar to *V. fauriei* than to *V. unijuga* (Fig. 4). This fact may mean that *V. bifolia* is phylogenetically closer to *V. fauriei* than to *V. unijuga*.

### Conclusion

The phylogenetic relationships among the East Asian species of *Vicia* presumed from electrophoretic patterns of seed proteins are almost entirely consistent with those with the recent morphological or DNA data. We consider that the electrophoretic patterns of seed proteins are useful to clarify inter- and intra-specific phylogenetic relationships among the East Asian endemic taxa of *Vicia*.

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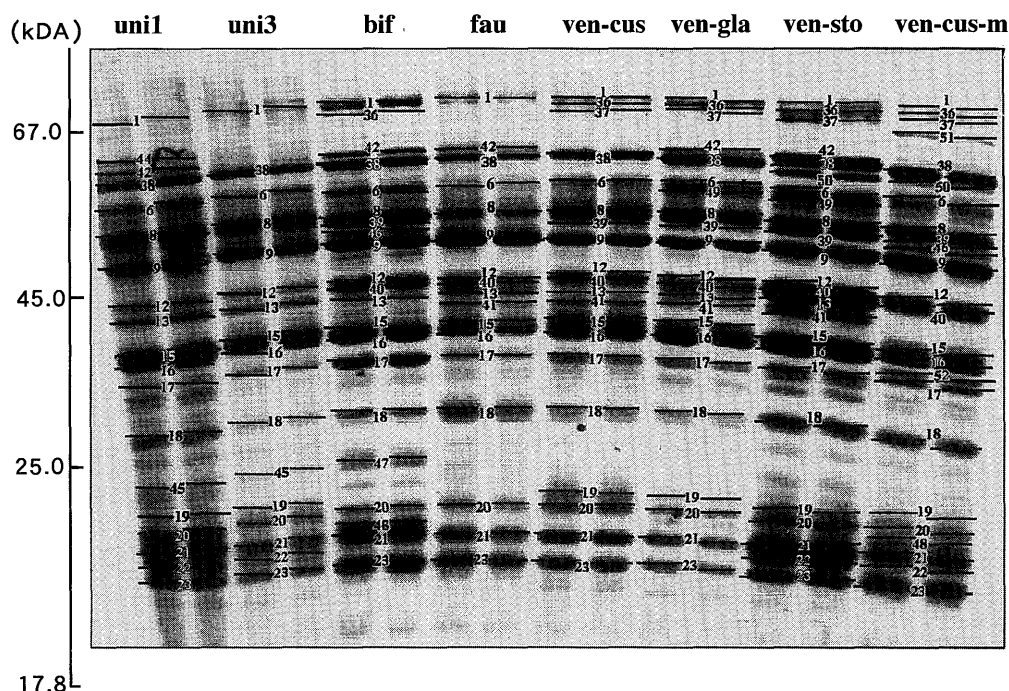


Fig. 3. SDS-PAGE patterns of seed proteins illustrating band patterns detected from eight accessions of *Vicia*. The abbreviations of accessions are designated in Table 1.

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Table 2. Presence (+) or absence (–) of seed protein bands in the accessions of *Vicia* examined

| No. <sup>b</sup> | Accessions <sup>a</sup> |            |     |       |       |       |     |     |             |            |             |               |       |       |       |       |       |     |     |
|------------------|-------------------------|------------|-----|-------|-------|-------|-----|-----|-------------|------------|-------------|---------------|-------|-------|-------|-------|-------|-----|-----|
|                  | ps-or<br>1              | ps-or<br>2 | jap | amo 1 | amo 2 | amo 3 | amu | nip | ven-<br>cus | ven-<br>gl | ven-<br>sto | ven-<br>cus-m | uni 1 | uni 2 | uni 3 | uni 4 | uni 5 | bif | fau |
| 1                | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 2                | +                       | +          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 3                | +                       | +          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 4                | +                       | +          | +   | +     | +     | +     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 5                | +                       | +          | -   | +     | +     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 6                | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 7                | +                       | +          | +   | +     | +     | +     | +   | +   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 8                | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 9                | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 10               | +                       | +          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 11               | +                       | +          | +   | +     | +     | +     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 12               | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 13               | +                       | +          | +   | +     | +     | +     | -   | +   | +           | +          | +           | -             | +     | +     | +     | +     | +     | +   | +   |
| 14               | +                       | +          | -   | -     | -     | -     | -   | +   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 15               | +                       | +          | +   | +     | +     | +     | +   | -   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 16               | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 17               | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 18               | +                       | +          | -   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 19               | +                       | +          | +   | +     | +     | +     | -   | -   | +           | +          | +           | +             | +     | +     | +     | +     | +     | -   | -   |
| 20               | +                       | +          | -   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 21               | +                       | +          | -   | +     | +     | +     | -   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 22               | +                       | +          | -   | -     | -     | -     | +   | +   | -           | -          | +           | +             | +     | +     | +     | +     | +     | -   | -   |
| 23               | +                       | +          | +   | +     | +     | +     | +   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 24               | -                       | -          | -   | +     | +     | +     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 25               | -                       | -          | +   | +     | +     | +     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 26               | -                       | -          | -   | +     | +     | +     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 27               | -                       | -          | -   | +     | +     | +     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 28               | -                       | -          | -   | +     | +     | +     | +   | +   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 29               | -                       | -          | -   | -     | -     | -     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 30               | -                       | -          | -   | -     | -     | -     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 31               | -                       | -          | +   | -     | -     | -     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 32               | -                       | -          | -   | -     | -     | -     | +   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 33               | -                       | -          | +   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 34               | -                       | -          | +   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 35               | -                       | -          | +   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 36               | -                       | -          | -   | -     | -     | -     | -   | -   | +           | +          | +           | +             | -     | -     | -     | -     | -     | +   | -   |
| 37               | -                       | -          | -   | -     | -     | -     | -   | -   | +           | +          | +           | +             | -     | -     | -     | -     | -     | -   | -   |
| 38               | -                       | -          | -   | -     | -     | -     | -   | +   | +           | +          | +           | +             | +     | +     | +     | +     | +     | +   | +   |
| 39               | -                       | -          | -   | -     | -     | -     | -   | -   | +           | +          | +           | +             | -     | -     | -     | -     | -     | +   | -   |
| 40               | -                       | -          | -   | -     | -     | -     | -   | -   | +           | +          | +           | +             | -     | -     | -     | -     | -     | +   | +   |
| 41               | -                       | -          | -   | -     | -     | -     | -   | -   | +           | +          | +           | -             | -     | -     | -     | -     | -     | -   | +   |
| 42               | -                       | -          | -   | -     | -     | -     | -   | +   | -           | +          | +           | -             | +     | +     | -     | -     | -     | +   | +   |
| 43               | -                       | -          | -   | -     | -     | -     | -   | +   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |
| 44               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | +     | +     | -     | -     | -     | -   | -   |
| 45               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | +     | +     | +     | +     | +     | -   | -   |
| 46               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | +             | -     | -     | -     | -     | -     | +   | -   |
| 47               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | -             | -     | -     | -     | -     | -     | +   | -   |
| 48               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | +             | -     | -     | -     | -     | -     | +   | -   |
| 49               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | +          | +           | -             | -     | -     | -     | -     | -     | -   | -   |
| 50               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | +           | +             | -     | -     | -     | -     | -     | -   | -   |
| 51               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | +             | -     | -     | -     | -     | -     | -   | -   |
| 52               | -                       | -          | -   | -     | -     | -     | -   | -   | -           | -          | -           | +             | -     | -     | -     | -     | -     | -   | -   |
| 53               | -                       | -          | -   | -     | -     | -     | -   | +   | -           | -          | -           | -             | -     | -     | -     | -     | -     | -   | -   |

Accessions<sup>a</sup>: The abbreviations of accessions are designated in Table 1.No.<sup>b</sup>: Number of bands indicated in Figs. 1–3.

Table 3. Jaccard's similarity matrix, based on the seed protein banding patterns observed in the present study, between all possible pairs of 19 accessions from 12 taxa of *Vicia*. The abbreviations of accessions are designated in Table 1

|           | ps-or1 | ps-or2 | jap  | amol | amo2 | amo3 | amu  | nip  | ven-cus | ven-gla | ven-sto | ven-cus-m | uni1 | uni2 | uni3 | uni4 | uni5 | bif  | fau  |
|-----------|--------|--------|------|------|------|------|------|------|---------|---------|---------|-----------|------|------|------|------|------|------|------|
| ps-or1    | 1.00   |        |      |      |      |      |      |      |         |         |         |           |      |      |      |      |      |      |      |
| ps-or2    | 1.00   | 1.00   |      |      |      |      |      |      |         |         |         |           |      |      |      |      |      |      |      |
| jap       | 0.50   | 0.50   | 1.00 |      |      |      |      |      |         |         |         |           |      |      |      |      |      |      |      |
| amol      | 0.64   | 0.64   | 0.55 | 1.00 |      |      |      |      |         |         |         |           |      |      |      |      |      |      |      |
| amo2      | 0.64   | 0.64   | 0.55 | 1.00 | 1.00 |      |      |      |         |         |         |           |      |      |      |      |      |      |      |
| amo3      | 0.60   | 0.60   | 0.57 | 0.95 | 0.95 | 1.00 |      |      |         |         |         |           |      |      |      |      |      |      |      |
| amu       | 0.48   | 0.48   | 0.50 | 0.53 | 0.53 | 0.55 | 1.00 |      |         |         |         |           |      |      |      |      |      |      |      |
| nip       | 0.53   | 0.53   | 0.34 | 0.48 | 0.48 | 0.50 | 0.48 | 1.00 |         |         |         |           |      |      |      |      |      |      |      |
| ven-cus   | 0.48   | 0.48   | 0.39 | 0.48 | 0.48 | 0.50 | 0.37 | 0.48 | 1.00    |         |         |           |      |      |      |      |      |      |      |
| ven-gla   | 0.45   | 0.45   | 0.36 | 0.45 | 0.45 | 0.46 | 0.35 | 0.50 | 0.90    | 1.00    |         |           |      |      |      |      |      |      |      |
| ven-sto   | 0.46   | 0.46   | 0.34 | 0.42 | 0.42 | 0.43 | 0.37 | 0.51 | 0.83    | 0.91    | 1.00    |           |      |      |      |      |      |      |      |
| ven-cus-m | 0.42   | 0.42   | 0.30 | 0.38 | 0.38 | 0.39 | 0.37 | 0.41 | 0.69    | 0.64    | 0.71    | 1.00      |      |      |      |      |      |      |      |
| uni1      | 0.55   | 0.55   | 0.40 | 0.50 | 0.50 | 0.51 | 0.44 | 0.62 | 0.62    | 0.64    | 0.65    | 0.53      | 1.00 |      |      |      |      |      |      |
| uni2      | 0.55   | 0.55   | 0.40 | 0.50 | 0.50 | 0.51 | 0.44 | 0.62 | 0.62    | 0.64    | 0.65    | 0.53      | 1.00 | 1.00 |      |      |      |      |      |
| uni3      | 0.60   | 0.60   | 0.44 | 0.53 | 0.53 | 0.56 | 0.48 | 0.60 | 0.68    | 0.62    | 0.64    | 0.57      | 0.89 | 0.89 | 1.00 |      |      |      |      |
| uni4      | 0.60   | 0.60   | 0.44 | 0.53 | 0.53 | 0.56 | 0.48 | 0.60 | 0.68    | 0.62    | 0.64    | 0.57      | 0.89 | 0.89 | 1.00 | 1.00 |      |      |      |
| uni5      | 0.60   | 0.60   | 0.44 | 0.53 | 0.53 | 0.56 | 0.48 | 0.60 | 0.68    | 0.62    | 0.64    | 0.57      | 0.89 | 0.89 | 1.00 | 1.00 | 1.00 |      |      |
| bif       | 0.41   | 0.41   | 0.33 | 0.41 | 0.41 | 0.43 | 0.36 | 0.51 | 0.70    | 0.72    | 0.66    | 0.66      | 0.60 | 0.60 | 0.58 | 0.58 | 0.58 | 1.00 |      |
| fau       | 0.48   | 0.48   | 0.38 | 0.48 | 0.48 | 0.50 | 0.42 | 0.60 | 0.76    | 0.77    | 0.70    | 0.51      | 0.71 | 0.71 | 0.70 | 0.70 | 0.70 | 0.72 | 1.00 |

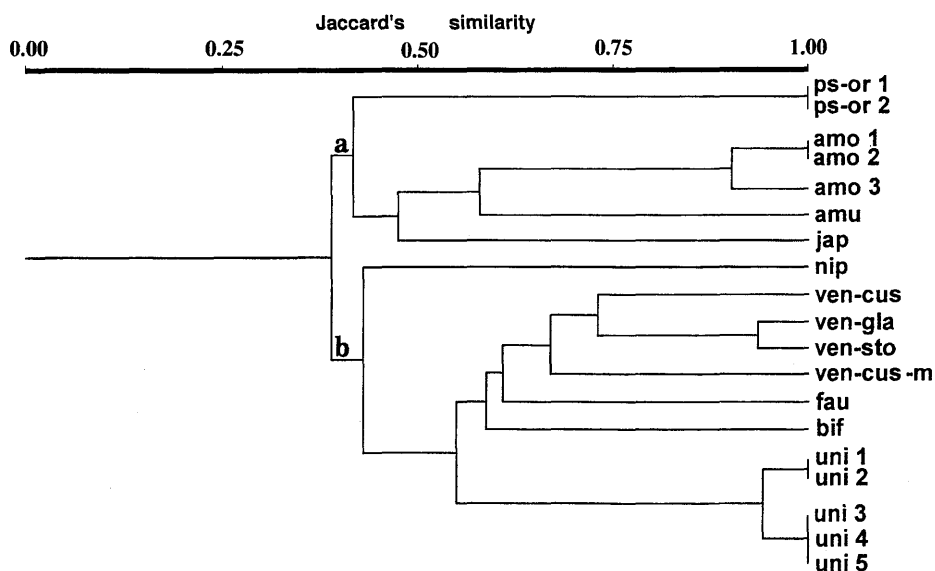


Fig. 4. UPGMA dendrogram of 19 accessions of 12 taxa of East Asian species of *Vicia* based on Jaccard's similarity matrix of electrophoretic patterns of seed proteins. The abbreviations of accessions are designated in Table 1.



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東アジア産ソラマメ属の種子蛋白質の電気泳動パ  
ターンとその分類学的意義

東アジアに固有に分布するマメ科ソラマメ属のうち、互いに近縁であると考えられている9種12分類群（ツルフジバカマ、ノハラクサフジ、ミヤマタニワタシ、ツガルフジ、ヒロハクサフジ、ヨツバハギ、オオバクサフジ、ナンテンハギ、および、エビラフジとその種内分類群の関係にあるシロウマエビラフジ、ビワコエビラフジ、ヒメヨツバハギ）について、相互の系統関係を推定するため、種子蛋白質の電気泳動パターンを解析した。電気泳動の結果、合計53本のバンドを認めた。これらバンドの有無により、分類群間のジャッカドの類似度を求め、これに基づき平均距離法により樹状図を作成した。そして、この樹状図から、分類群間の系統関係を推定した。

ミヤマタニワタシは、ナンテンハギよりもエビラフジに近縁であると推定された。このことは、DNA データを用いて行われた既存の推定に一致し、これを支持する結果となった。一方、ビワコエビラフジ（エビラフジの別亜種）はヒメヨツバハギ（エビラフジの別品種）よりもエビラフジに近縁となり、これら種内分類群の分類の再検討の必要性が示された。

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